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TOXICITY OF GALACTOSE FOR CERTAIN OF THE HIGHER PLANTS[1]

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In the course of investigations upon the effect of sugars on the growth of certain higher plants, the sugar galactose was employed. In experiments with vetch (*Vicia villosa*) the plants grown in the presence of 2 per cent galactose showed very marked injury, the injury being especially manifest by a killing of the roots and accompanied by a reduction in the growth of tops. The results secured were the more surprising in view of the fact that lactose sugar employed coincidently influenced beneficially the growth of the same plant. Certain experiments were therefore made to determine whether or not the effect of the galactose was consistent.

Method of experimentation.—The plants were grown under sterile conditions on agar media containing Pfeffer's nutrient solution[2] of one-half its normal strength. This solution is neutral in its reaction. The solution contained varying amounts of galactose sugar, the source of which is indicated in each case.

[1] The writer acknowledges gladly his indebtedness to the officers of the Missouri Botanical Garden for facilities and courtesies extended to him during his stay in St. Louis.

[2]

$CaNO_3$	2	grams
KNO_3	0.5	grams
KCl	0.25	grams
K_2HPO_4	0.50	grams
$MgSO_4$	0.50	grams
Fe_2Cl_3	4	milligrams
Dist. water	6	liters

The seed employed were sterilized by means of a method devised in the Laboratory of Plant Physiology of Cornell University by Dr. J. K. Wilson.[1] In brief it is as follows: 10 grams of chloride of lime are shaken up with 150 cc. tap water and after standing for ten minutes the supernatant liquid is filtered. The filtrate is used as the sterilizing agent. The seeds are placed in a test-tube covered with about five times their volume of the filtrate and the tube then tightly stoppered. The seeds are treated for from 4 to 24 hours, depending upon the character of the seed. In the experiments here mentioned the vetch seeds were exposed to this treatment for 12 hours and the peas for 4 hours. The seeds are directly transferred to the culture vessels from the chloride of lime solution, care being observed to drain off all of the chloride of lime solution. In transferring the seed the usual bacteriological precautions are observed.

Experiment with vetch (*Vicia villosa*).—The plants were grown in large glass cylinders 60 cm. high and 10 cm. in diameter, having a volume of approximately 4 liters. In each of the cylinders were placed 250 cc. of the nutrient solution plus 1 per cent washed agar and galactose sugar. The cylinders were then fitted with cotton plugs and sterilized for one hour in an autoclave at a pressure of 15 pounds. The cultures were made in triplicate and the galactose was tested at 2 per cent and at 0.2 per cent concentration. After a growth period of 30 days the cultures showed the injurious action of the galactose, in each case the roots being markedly injured. The primary root tip coming in contact with the agar medium was killed and the lateral root produced met with the same injury, so that ultimately a multi-branched root system was produced after the manner of the pea roots shown in pl. 22 fig. 5. Whatever portions of the roots remained in contact with the agar medium were ultimately killed. It should be mentioned in this connection that the vetch grown in the presence of glucose, saccharose, lactose or maltose at concentrations of 2 per cent was greatly benefited. These sugars are absorbed and assimilated.

[1] Am. Jour. Bot. **2:** 420–427. 1915.

Experiment with Canada field pea (Pisum sativum).—In the first experiment with the pea the large cylinders were again employed and to each were added 200 cc. of the nutrient solution plus 1 per cent agar and the sugar whose effect was to be tested. Cultures were made with raffinose, saccharose, lactose, glucose, and galactose ("Merck's Highest Purity"), the concentration of the sugar employed in each case being 2 per cent. The cylinders were fitted with cotton plugs as in the previous experiment and then sterilized for a period of one hour. In each cylinder were sown four peas which had

TABLE I

DATA ON CANADA FIELD PEA

(Duration 25 days. Taken February 13)

Culture	No. of plants	Height of plants cm.	Total green wt. grams	Dry wt. coty-ledons grams	Dry wt. roots grams	Dry wt. tops grams	Total dry wt. grams	Av. dry wt. grams	Gain per plant grams
Glucose	3	44 40 38	6.250	.155	.170	.364	.689	.229	+.085
Lactose	4	40 40 33 33	6.700	.169	.105	.355	.629	.157	+.007
*Raffinose	4	32 33 24 33	6.500	.192	.130	.328	.650	.162	+.012
Saccharose	4	39 35 36 35	7.600	.160	.144	.430	.734	.183	+.036
Check	3	32 23 34	4.450	.150	.075	.190	.415	.138	—.012
Maltose	4	33 40 34 28	6.600	.222	.142	.386	.750	.187	+.034
Galactose	Plants small and roots injured. (See pl. 22, figs. 1a and 5.)								

* Reducing sugar formed in medium probably as a result of secretion of invertase and raffinase from roots. Acidity of entire medium at time of examination equivalent to 0.7 cc. N/10 KOH.

been sterilized by the method described. The plants were grown for a period of twenty-five days and then data taken on the various cultures. The various cultures are shown in pl. 22 fig. 1. The galactose plants are separately shown in pl. 22 fig. 5 and the detailed data are given in table I.

An examination of the table reveals the fact that every sugar acted beneficially except galactose. If the plants had been examined a month later (as was the case with other cultures), much greater differences would have been secured between the check cultures and the sugar-containing cultures. Lactose is undoubtedly utilized by Canada field pea as well as by vetch and probably before assimilation is converted into glucose and galactose. Raffinose, which is also utilized, yields on hydrolysis first levulose and melibiose, and the latter is further transformed to galactose and dextrose. In the light of the foregoing, it would appear from the results secured with lactose and raffinose that levulose and glucose must exert some protective action against the injurious action of galactose.

Influence of concentration of galactose.—In all of the previous experiments the galactose sugar was employed at only two concentrations, namely, 0.2 per cent and 2 per cent. In the following experiment a series of cultures was made containing galactose at the following concentrations: 0.125 per cent, 0.25 per cent, 0.50 per cent, 1.0 per cent, 2.0 per cent, and control cultures lacking galactose. The plants were grown in large test-tubes 30 cm. × 4 cm., containing 50 cc. of the nutrient medium plus 1 per cent agar. The galactose sugar employed in this experiment was provided by Dr. C. S. Hudson[1], Chief of the Carbohydrate Laboratory, U. S. Bureau of Chemistry. The galactose sugar provided had been recrystallized and was stated by Dr. Hudson to be of a very high degree of purity and probably purer than any which could be secured upon the market. The tubes were plugged with cotton and sterilized in an autoclave at 15 pounds pressure for a period of 20 minutes. One pea was sown in each tube and the cul-

[1] The writer gratefully acknowledges his indebtedness to Dr. Hudson for the galactose furnished.

tures made in triplicate. The seeds germinated in four days and even by this time in the higher concentrations of galactose, browning of the cotyledons was becoming evident. This browning of the cotyledons intensified with time and at the end of 20 days the peas in the 1 per cent and 2 per cent galactose cultures showed marked discoloration, and death of roots soon occurred. The height of tops was also markedly affected in the presence of galactose of a concentration of 1 per cent or over. (See pl. 22 fig. 4.) The above experiments were repeated with wheat and corn and the results secured were similar.

Antagonistic action of glucose toward toxicity of galactose.—It was noted previously that raffinose and lactose are utilized by Canada field pea, and this has been verified by other experiments. The use of lactose by vetch has also been decidedly shown by experiments not yet reported. Since both lactose and raffinose are assimilated by pea and vetch, and since it is highly probable, as previously suggested, that these sugars are hydrolyzed before assimilation, it is possible that the glucose and levulose exercise a protective action against the galactose.

An experiment was made to test the hypothesis with respect to glucose. Test-tube cultures were prepared as in the previous experiment, but in this case were made in quadruplicate. One series contained 1 per cent glucose plus 1 per cent galactose and the second series contained 1 per cent galactose alone. The plants were grown for 25 days in the greenhouse and the general results are clearly evident in pl. 22 figs. 2 and 3. In the case of the 1 per cent galactose culture the primary roots were killed, but with the 1 per cent glucose added, the primary root tip was killed and the epidermis and part of the cortex, but the inner part of the root was not apparently injured, for secondary roots developed which seemed to be more resistant to the toxic action of the galactose, for these root tips suffered no injury and not even a browning of the root was secured as was the case with the primary root (pl. 22 fig. 3). The experiment was repeated a second time and the results secured are concordant with the first.

Discussion.—So far as the writer has been able to discover, no previous mention has been made of the toxic nature of galactose for plants. Molliard[1], however, intimates that galactose is toxic for radish, for unlike other sugars, the galactose permitted no development beyond a 5 per cent concentration and with 2 per cent galactose the plants are very small. He concludes that galactose is not utilized by radish.

That galactose is injurious to the green plants employed is definitely shown. It does not appear to be toxic to fungi since *Aspergillus niger,* several species of *Penicillium,* a species of *Fusarium,* and a species of *Mucor* were all found growing in cultures which became contaminated. It is definitely known also that certain yeasts are able to ferment galactose. The character of the injury effected by the galactose in the above experiment and the method of action have not yet been determined. Incidental observations indicate that the galactose on penetrating kills the cells in its path. In the case of peas grown on 1 per cent galactose the peripheral layers of the cotyledon showed the original starch reserve undigested. In the presence of glucose it was only the epidermis and part of the cortex which suffered injury. It would appear that the outer layers of cells were injured before sufficient glucose had accumulated to render them resistant to the toxic action of galactose, or perhaps the penetrability of the inner cells for galactose was altered by the presence of glucose. In what manner the glucose antidotes the toxicity of galactose cannot yet be stated. It may be possible that it is the oxidation products of galactose that are the injurious agents and that the glucose prevents the formation, or modifies the character, of the oxidation products and that the toxicity is thereby overcome. Investigation into other phases is in progress.

[1] Molliard, Marin. Action morphogénique de quelques substances organiques sur les végétaux supérieurs. Rev. Gén. de Bot. **19**: p. 331. 1907.

Explanation of Plate

PLATE 22

Fig. 1. *a*, galactose 2 per cent; *b*, lactose 2 per cent; *c*, check—no sugar; *d*, raffinose 2 per cent; *e*, saccharose 2 per cent; *f*, glucose 2 per cent. (Cotton plugs were removed at the time of photographing.)

Fig. 2. The two outside tubes contain 1 per cent galactose while the two middle ones contain 1 per cent galactose plus 1 per cent glucose.

Fig. 3. The small plant was grown on 1 per cent galactose; the larger one on 1 per cent galactose plus 1 per cent glucose.

Fig. 4. *a* and *b*, 2 per cent galactose; *c*, 1 per cent galactose; *d*, 0.500 per cent galactose; *e*, 0.250 per cent galactose; *f*, 0.125 per cent galactose; *g*, check—no galactose.

Fig. 5. Peas (shown in fig. 1*a*), showing character of root growth when tips alone come in contact with 2 per cent galactose-containing medium.

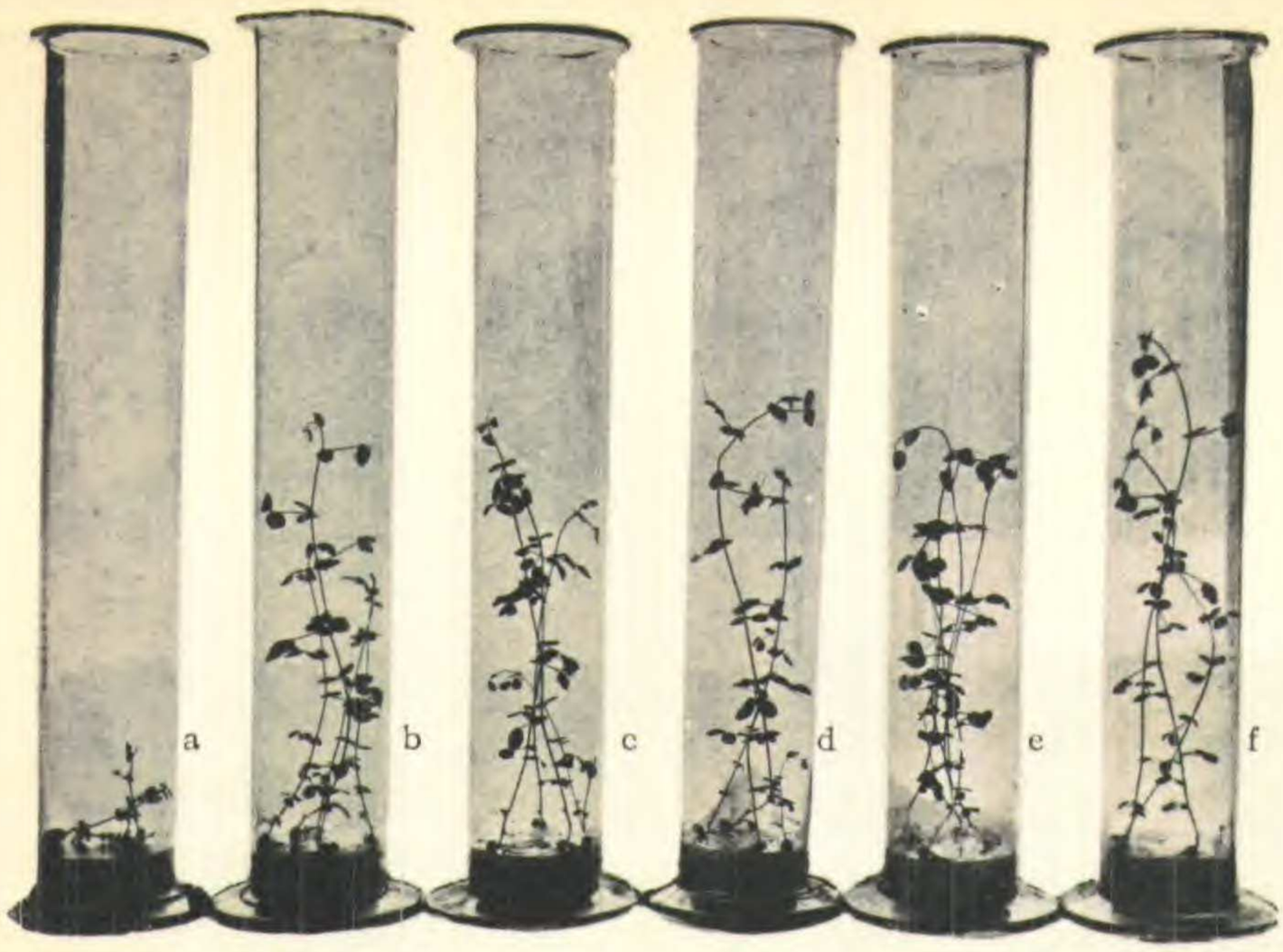

Fig. 1

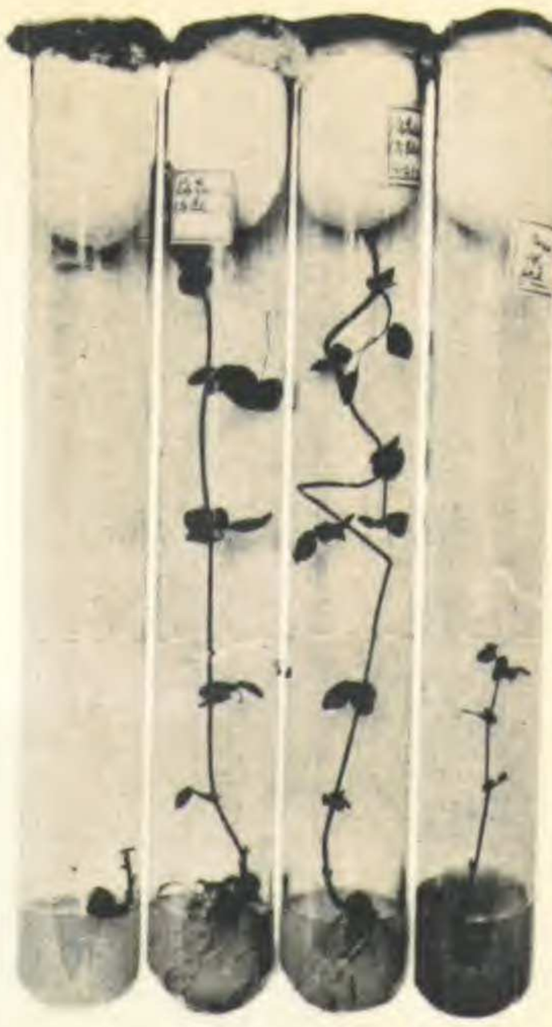

Fig. 2

Fig. 3

Fig. 4

Fig. 5

KNUDSON—TOXICITY OF GALACTOSE

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